

Effect of Chlorine on *Giardia lamblia* Cyst Viability

EDWARD L. JARROLL,[†]* ALAN K. BINGHAM, AND ERNEST A. MEYER

Department of Microbiology and Immunology, University of Oregon Health Sciences Center,
Portland, Oregon 97201

The effect of chlorine concentration on *Giardia lamblia* cyst viability was tested under a variety of conditions. The ability of *Giardia* cysts to undergo excystation was used as the criterion of viability. The experimental variables employed included temperature (25, 15, and 5°C), pH (6, 7, and 8), chlorine-cyst contact time (10, 30, and 60 min), and chlorine concentration (1 to 8 mg/liter). In the pH range studied, cyst survival generally was observed to increase as buffer pH increased. Water temperature coupled with chlorination proved to be important in cyst survival. Results of these experiments at the three temperatures studied can be summarized as follows: at 25°C, exposure to 1.5 mg/liter for 10 min killed all cysts at pH 6, 7, and 8. At 15°C, 2.5 mg of chlorine per liter for 10 min killed all cysts at pH 6, but at pH 7 and 8 small numbers of cysts remained viable after 30 min but not after 60 min. At 5°C, 1 mg of chlorine per liter for 60 min failed to kill all the cysts at any pH tested. At this temperature, 2 mg of chlorine per liter killed all cysts after 60 min at pH 6 and 7, but not at pH 8. A chlorine concentration of 4 mg/liter killed all the cysts at all three pH values after 60 min, but not after 30 min. A chlorine concentration of 8 mg/liter killed all *Giardia* cysts at pH 6 and 7 after contact for 10 min, and at pH 8 after 30 min. This study points up the role of temperature, pH, and chlorine demand in the halogen treatment of drinking water to destroy cysts. It also raises an epidemiological problem, namely: low water temperatures, where killing of *Giardia* requires relatively high chlorine concentrations and long contact times, are (i) to be expected in many areas where epidemic waterborne giardiasis has been reported and (ii) particularly conducive to the long-term survival of *Giardia* cysts.

Parasitic flagellates in the genus *Giardia* are distributed worldwide and are the most commonly reported human intestinal parasites in the United States and Britain (15). Host-to-host transmission of *Giardia* occurs when viable cysts are ingested directly or in food or water contaminated with feces. In recent years, it has become evident that giardiasis can be spread in epidemic form among humans in temperate and cold climates and that the vehicle of spread is drinking water (15). Waterborne giardiasis has been reported in the United States from New York (20), New Hampshire (13), Pennsylvania (18), Colorado (14, 16-18, 23), California (18), Utah (1), Oregon (18, 22), and Washington state (7).

No guidelines exist for the chemical treatment of public drinking water to destroy *Giardia* cysts because little is known of the effect of halogens on *Giardia* cyst viability. Hoff (10) summarized the early literature dealing with the effect of chlorine on *Giardia*. He noted that a misinter-

pretation of a study which stated that 0.5% chlorinated water killed *G. intestinalis* (syn. *G. lamblia*, the species parasitic in humans) and *Entamoeba histolytica* cysts in 2 to 3 days resulted in a statement that 0.5% chlorinated water would not kill protozoan cysts in that length of time. Hoff (10) suggested that this misunderstanding accounts for the belief that *G. lamblia* cysts are extremely resistant to chlorine.

In two recent reports, six halogen methods used for disinfecting small quantities of water were assessed for their ability to destroy *G. lamblia* cysts; several of these methods proved unsatisfactory, especially in cold water (11, 12). Those methods which failed to destroy *G. lamblia* cysts completely were unsatisfactory because of insufficient halogen residuals or contact times and not because of an extreme resistance of the cysts to the halogens. Although these studies showed that very high levels of chlorine and iodine will kill *G. lamblia* cysts, they did not involve the study of the dynamics of the process. Thus, the controversy continues regarding the adequacy (4, 8, 10) or the practicality of standard bactericidal chlorination (9) of drinking

[†] Present address: Department of Preventive Medicine, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14850.

water for killing *G. lamblia* cysts.

Therefore, it is the intent of this paper to report the results of experiments which tested the effect of various concentrations of free chlorine on *G. lamblia* cyst survival.

MATERIALS AND METHODS

G. lamblia cysts used in these experiments were concentrated and purified (2) from several human fecal specimens and stored in deionized water at 3°C. Cyst numbers were determined by counts with a hemocytometer.

Sørensen sodium phosphate buffer, consisting of 0.2 M monobasic and 0.2 M dibasic sodium phosphate, was made chlorine demand-free by adding 100 μ l of 5% sodium hypochlorite solution (NaOCl) to 1,000 ml of the buffer solutions, followed by the exposure of these buffers to 24 h of ultraviolet irradiation to decompose the excess chlorine (19). The buffer was mixed in proportions that yielded solutions of pH 6, 7, and 8 and diluted with demand-free water to a 0.01 M concentration, which held the pH constant and was not detrimental to the cysts.

Chlorine stock solutions were made by adding sodium hypochlorite to the diluted demand-free buffer. Chlorine residuals were determined by titration with a Wallace and Tiernan amperometric titrator (11).

A 250-ml amount of 0.01 M demand-free chlorinated buffer was added to each of three 800-ml glass beakers with covers, maintained at either 25, 15, or 5°C in a constant-temperature water bath, and mixed with a paddle stirrer. A cyst suspension was added to each beaker to a final concentration of approximately 650 cysts per ml. The chlorine demand of each cyst preparation was predetermined and ranged from as low as 0 to 0.1 mg/liter. When the cyst suspension was reduced in volume and purified as much as possible and the chlorine demand remained above zero, additional chlorine was added to compensate for the cyst demand.

After a contact time of 10, 30, or 60 min, a 20-ml sample of each solution was removed and diluted to 200 ml with demand-free water, and the chlorine residual was verified. Simultaneously, 47 ml of each solution was removed and added to 3 ml of a 0.6% solution of sodium thiosulfate to stop the action of the chlorine. The latter mixture was centrifuged at 600 \times g for 5 min, and the resulting cyst pellet was exposed to an excystation procedure (3). Controls were cysts suspended in 0.01 M Sørensen phosphate buffer without chlorine but otherwise treated the same as the chlorine solutions. The experimental protocols involved simultaneously conducting buffer and thiosulfate controls at each temperature, pH, and contact time as the chlorine treatments. Data from representative controls are included in the Results section.

Control excystation ranged from 35 to 65% depending on the day that the experiments were conducted. Based on this range of excystation, detection of $\geq 0.2\%$ cyst survival was the lower limit of sensitivity for this counting procedure.

Disinfection experiments were performed in duplicate (triplicate where the quantity of cysts allowed) by counting at least 1,000 cysts at each trial, and the

percentage of excystation was determined (2). The results were expressed as percent cyst survival and were calculated as follows: control excystation (cysts in buffer without chlorine) was designated 100%, and the percentage of excystation after exposure to chlorinated buffer was expressed relative to the control.

RESULTS

Virtually no differences in the levels of excystation of *G. lamblia* were found regardless of the contact time in nonchlorinated buffer (Table 1). Additionally, the pH of the buffer apparently had little effect on the survival of the *G. lamblia* cysts even after a 60-min exposure (Table 2). The sodium thiosulfate, as has been reported earlier (11), had no effect on *G. lamblia* cyst survival at the concentration used in these experiments.

At 25°C, there was no detectable survival of *G. lamblia* cysts which had been exposed to chlorine concentrations of 1.5 mg/liter or higher for 10 min at pH 6, 7, or 8 (Fig. 1).

At 15°C (Fig. 2), no cysts survived a 10-min exposure to 2.5 mg of chlorine per liter in buffer at pH 6, but 1.8% of the cysts survived for 10 min and 0.4% survived for 30 min in buffer at pH 7. In buffer at pH 8 at this chlorine concen-

TABLE 1. Mean percentage of excystation of *G. lamblia* cysts in 0.01 M Sørensen phosphate buffer at pH 7 after 0, 10, 30, and 60 min of exposure at 25, 15, and 5°C^a

Temp (°C)	Mean % excystation (\pm standard error) at exposure:			
	0 min	10 min	30 min	60 min
25	50.8 \pm 5.2	45.6 \pm 4.1	44.8 \pm 3.7	48.5 \pm 6.4
15	56.2 \pm 3.1	50.8 \pm 6.3	51.4 \pm 1.7	51.6 \pm 2.6
5	45.1 \pm 2.6	43.5 \pm 2.8	44.4 \pm 3.1	48.3 \pm 2.8

^a These data are representative of those obtained at pH 6 and 8. These data were analyzed by a one-way analysis of variance, and the zero mean for a given temperature is not significantly different ($P > 0.05$) from the other means at that temperature.

TABLE 2. Mean percentage of excystation of *G. lamblia* cysts after 60 min in Sørensen phosphate buffer at pH 6, 7, and 8^a

Temp (°C)	Mean % excystation (\pm standard error) at pH:		
	6	7	8
25	52.6 \pm 9.8	55.4 \pm 6.6	53.2 \pm 9.5
15	45.1 \pm 7.6	48.5 \pm 6.7	49.9 \pm 5.3
5	49.3 \pm 7.9	49.4 \pm 9.9	50.2 \pm 6.6

^a These data are representative of those obtained at times of 0, 10, and 30 min. These data were analyzed by a one-way analysis of variance and at a given temperature the means are not significantly different ($P > 0.05$) from each other.

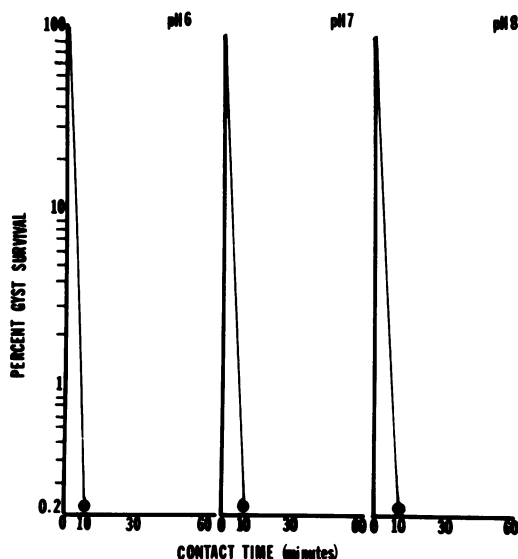


FIG. 1. Representative *G. lamblia* cyst survival at 25°C in 1.5 mg of chlorine per liter after 10, 30, and 60 min of contact time in buffer at pH 6, 7, and 8. Plotted points are means of at least two trials.

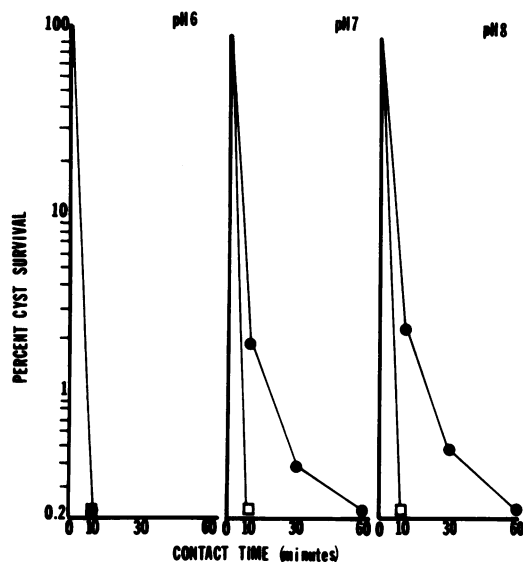


FIG. 2. Representative *G. lamblia* cyst survival at 15°C in two concentrations of chlorine after 10, 30, and 60 min of contact time in buffer at pH 6, 7, and 8. Plotted points are means of at least two trials. Chlorine concentrations shown are 2.5 mg/liter (●) and 3.0 mg/liter (□).

tration and temperature, cyst survivals of 2.0 and 0.4% were noted after 10 and 30 min, respectively. No cysts survived a 60-min exposure to 2.5 mg/liter at pH 7 or 8. No cyst survival was observed after a 10-min exposure to 3.0 mg of

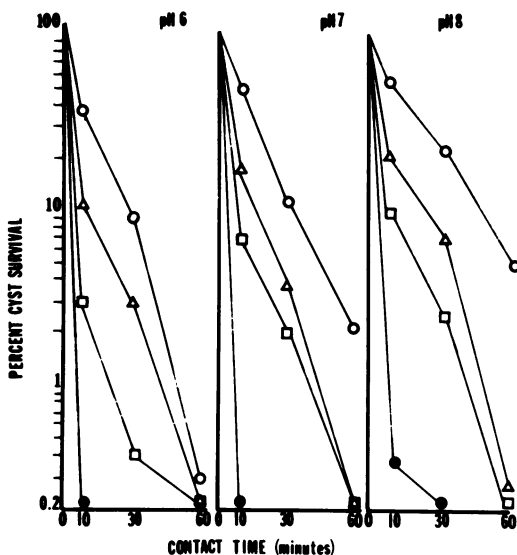


FIG. 3. Representative *G. lamblia* cyst survival at 5°C in various concentrations of chlorine after 10, 30, and 60 min of contact time in buffer at pH 6, 7, and 8. Plotted points are means of at least two trials. Chlorine concentrations are 1 mg/liter (○), 2 mg/liter (△), 4 mg/liter (□) and 8 mg/liter (●).

chlorine per liter at any of the pH values tested.

At 5°C (Fig. 3), cyst survival in general increased as the pH of the buffer increased. In buffer containing 1 mg of chlorine per liter, cyst survival after 10 min ranged from a low of 35% at pH 6 to a high of 56% in buffer at pH 8.

Regardless of the pH of the buffer at 5°C, cyst survival decreased by approximately half after 10 and 30 min at a chlorine concentration of 2 mg/liter and decreased by half again at a chlorine concentration of 4 mg/liter. Virtually no cyst survival was detected at either 2 or 4 mg of chlorine per liter after 60 min at this temperature regardless of the pH of the buffer.

At 8 mg of chlorine per liter (Fig. 3), none of the *G. lamblia* cysts survived a 10-min exposure in buffer at either pH 6 or 7, but 0.4% of the cysts survived for 10 min at pH 8. After 30 min, no survival was observed in 8 mg of chlorine per liter even at pH 8.

DISCUSSION

G. lamblia cysts survived equally well in non-chlorinated control buffers for up to 60 min regardless of the temperature or the pH tested. In an earlier study of *G. lamblia* excystation (2), cysts survived for about 1 month in water stored at 21°C and for up to 2 months in water at 8°C. Thus, it is not surprising that the relatively short exposure to the temperatures used in this study

had no apparent detrimental effect on cyst survival.

The chlorine concentrations that were shown in this study to be cysticidal for *G. lamblia* at 25 and 15°C were similar to those that were cysticidal for *E. histolytica* (5, 6, 21). It is likely that additional studies, had the cyst supply permitted, would have shown that shorter contact times and lower chlorine concentrations are cysticidal for *G. lamblia* at 25°C. No data comparable to that for *G. lamblia* at 5°C appear to exist for *E. histolytica* cysts.

The higher chlorine concentrations and longer contact times required to destroy *G. lamblia* cysts at low temperatures were expected (11). Chang and Fair (6) showed that increasing the water temperature above 10°C decreased *E. histolytica* cyst survival at a given chlorine concentration and decreased the cysticidal concentration of chlorine required.

Also expected was the increased cysticidal efficacy of chlorine at low rather than at high pH. Chang and Fair (6) suggested that lower cysticidal concentrations of chlorine were required as the pH of the solution decreased. Stringer and Kruse (21), also using *E. histolytica* cysts, demonstrated that chlorine was a more effective cysticide at low rather than high pH because at low pH an effective cysticidal chlorine species, HOCl, predominates. A less effective chlorine species, OCl⁻, predominates at high pH. It should be noted that increasing the temperature of the buffer within the narrow range of pH tested in this study appeared to enhance the cysticidal action of the chlorine more dramatically than decreasing the pH.

Our results clearly demonstrate that viable *G. lamblia* cysts can be destroyed by chlorine and that this destruction can take place even at concentrations lower than those previously tested (11). Furthermore, the dynamics of chlorine disinfection of water containing *G. lamblia* cysts appear to be comparable to those of chlorine disinfection of water containing *E. histolytica* cysts. These studies emphasize that when chlorine is used as a cysticide against *G. lamblia*, attention should be given to the pH, chlorine demand, and temperature of the water being treated. The temperature consideration is especially important since: (i) halogens have a reduced cysticidal capacity in cold water (6, 11); (ii) *G. lamblia* cysts survive longer in cold than in warm water (2); and (iii) the water temperatures in many areas where epidemic giardiasis has been reported are likely to be cold even during the summer months.

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